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SUMMARY: SAFETY AND EFFECTIVENESS INFORMATION FOR AneuVysion® Multicolor DNA Probe Kit

The AneuVysion™ Multicolor DNA Probe Kit (AneuVysion kit) is a combination of two DNA probe mixtures; CEP 18/X/Y and LSI 13/21. The CEP 18/X/Y probe is a mixture of directly labeled fluorescent DNA probes specific for the D18Z1, DXZ1 and DYZ3 regions of chromosomes 18, X, and Y, respectively. The LSI 13/21 probe contains a mixture of unique DNA sequences that hybridize in the 13q14 region of chromosome 13, and unique DNA sequences complementary to the D21S259, D21S341, and D21S342 loci contained within the 21q22.13 to 21q22.2 region on the long arm of chromosome 21. The LSI 13 probe was created from a set of overlapping clones, which contain the entire RB-1 gene as well as regions extending beyond the gene on both sides. The probe extends beyond the 180 kb RB-1 gene for 110-170 kb in the 5' direction and approximately 120 kb in the 3' direction; the entire probe is 410-470 kb. CEP 18/X/Y is an aqua, green, and orange tri-color probe mixture and LSI 13/21 is a green and orange dual-color probe mixture.

The AneuVysion (CEP 18, X, Y-alpha satellite, LSI 13 and 21) Multicolor Probe Panel is intented to use CEP 18/X/Y probe to detect alpha satellite sequences in the centromere regions of chromosomes 18, X, and Y, and LSI 13/21 probe to detect the 13q14 region and the 21q22.13 to 21q22.2 region. The AneuVysion kit is indicated for identifying and enumerating chromosomes 13, 18, 21, X, and Y via fluorescence *in situ* hybridization (FISH) in metaphase cells and interphase nuclei obtained from amniotic fluid in subjects with presumed high risk pregnancies. It is not intended to be used as a stand-alone assay for making clinical decisions. FISH results are intended to be used as an aid in the diagnosis of numerical abnormalities of chromosomes 13, 18, 21 X and/or Y in conjunction with other information currently used in prenatal diagnosis, consistent with professional standards of practice [1]. This device is intended for use only with amniocyte cells; it is not intended for and has not been validated for use with other test matrices. This FISH assay will not detect the presence of structural chromosome abnormalities that can also result in birth defects. This FISH assay will be performed in cytogenetics laboratories.

Ref [1]: American College of Medical Genetics. Technical and clinical assessment of fluorescence in situ hybridization: An ACMG/ASHG position statement. I. Technical considerations. *Genetics in Medicine*. 2000;2(6):356-361.

Standard cytogenetic analysis detects cytogenetic abnormalities by karyotyping metaphase spreads after staining the chromosomes with a dye in cultured tissue cells.

Safety and effectiveness issues relevant to FISH assays such as the AneuVysion assay may include cross-reactivity, poor sensitivity, poor specificity, or poor reproducibility.

[1] American College of Medical Genetics. Technical and clinical assessment of fluorescence in situ hybridization: an ACMG/ASHG position statement. I. Technical considerations. *Genetics in Medicine*. 2000;2(6):356-361

Analytical Sensitivity and Specificity

Hybridization Efficiency

In the pivotal study, among the human amniotic fluid specimens, the average percentage of cells with no hybridization signal was 0.42% for LSI 13, CEP 18, and LSI 21. The average percentage of cells with only one hybridization Y signal was 0.06% for CEP X/Y. Thus, <2% cells with no or only one signal for each probe is a realistic standard of acceptance.

Analytical Sensitivity

The analytical sensitivity of the AneuVysion kit probes was tested in the reproducibility study described below. In that study, the 100% XY (or 0% X0) specimen was estimated with a mean of 0.04% (±0.2%), and the 10% X0 specimen was estimated with a mean of 9.10% (±1.79%) X-signaled nuclei. The upper 95% CI was 0.43 for the 0% X0 specimen and the lower 95% CI for the 10% X0 specimen was 5.59%. Thus, the limit of detection for the AneuVysion™ kit in interphase cells is estimated to be 3%.

Analytical Specificity

Locus specificity studies were performed with metaphase spreads according to standard Vysis QC protocols. A total of 705 metaphase spreads were examined sequentially by G-banding to identify chromosomes 13, 18, 21, X, and Y, followed by FISH. No cross-hybridization to other chromosome loci was observed in any of the 705 cells examined; hybridization was limited to the target regions of chromosomes 13, 18, 21, X, and Y.

Reproducibility

A pivotal study was conducted to assess the reproducibility of the AneuVysion™ assay interphase analysis for the percentage of aneuploid cells. The AneuVysion™ assay were assessed for inter-site, inter-lot, inter-day and inter-observer reproducibility. One normal and three mosaic cultured human amniocyte specimens were evaluated for the percentage of aneuploid cells according to the instructions for signal enumeration in the package insert. Using ANOVA, no significant variations were observed in any of the inter-assay reproducibility parameters. The intra-assay mean, S.D., and percent C.V. of the observed percentage of aneuploid nuclei for all samples are shown in Table 1.

Table 1
Precision of % Aneuploid Cells by Level of Mosaicism

Specimen	N	Summary Statistics	%X	%XX	%XXX	%XY	+ 21	%2-sig CH-21	%2-sig CH-18	%2-sig CH-13
100% XY	24	mean	0.04	0.04	0.00	98.88	0.79	96.7	94.6	97.1
		S.D.	0.20	0.20	0.00	1.14	0.77	1.01	1.98	1.53
		C.V. (%)	---	---	---	1.2	---	1.0	2.1	1.6
10% X/ 90% XX	24	mean	9.10	88.17	0.85	0.00	1.25	94.8	94.9	95.6
		S.D.	1.51	1.61	0.67	0.00	0.69	1.44	1.62	1.69
		C.V. (%)	16.6	1.8	---	---	---	1.5	1.7	1.8
17% X/ 47% XX/ 36% XYX	24	mean	19.48	42.56	36.68	0.00	1.16	96.1	95.7	97.3
		S.D.	4.06	3.65	3.74	0.00	0.72	1.72	1.16	1.03
		C.V. (%)	20.8	8.6	10.2	---	---	1.8	1.2	1.1
50% XY+21/ 50% XY	24	mean	0.04	0.00	0.00	98.13	52.03	45.98	96.1	97.1
		S.D.	0.12	0.00	0.00	1.34	3.25	3.69	0.90	1.33
		C.V. (%)	---	---	---	1.4	6.2	8.0	0.9	1.4

Methods Comparison: Clinical Specimens

A multi-center, blinded, controlled, comparative study was conducted to further define the performance characteristics of the AneuVysion™ kit relative to standard cytogenetic analysis, the standard of care, in cultured and uncultured amniotic fluid specimens. Thirty-one investigation sites analyzed amniotic fluid specimens obtained from a total of 1516 patients.

All study sites conducted the trial according to the prescribed assay procedures and signal enumeration guides.

A total of 2,238 amniocyte specimens were obtained and analyzed from 1,516 patients. Of these 2,238 specimens, 55 were deemed uninformative. These 55 uninformative specimens included three due to maternal cell contamination, four due to FISH assay failures and 48 had an insufficient (<40) number of nuclei for analysis. Thus on per specimen basis, the rate of informativeness is 97.5% (2183/2238). Note that among the 48 uninformative cases due to an insufficient (<40) number of nuclei available for one or both of the probes, 31 were partially uninformative for either the CEP 18/X/Y (22) or the LSI 13/21 (9).

Of the 1516 patients, thirteen patients with either cultured or uncultured specimens were included in the 55 uninformative specimens. Thus on per patient basis, the rate of informativeness is 99.8% (1503/1516). One specimen per patient was included in the primary analyses. Of these, 589 were cultured and 927 were uncultured specimens.

The maternal age ranged from 13 to 52 years, with a mean (±S.D.) age of 33.2 years (±6.8 years). The gestational age ranged from 11 to 38 weeks, with a mean (±S.D.) of 18.8 weeks (±4.6 weeks). The mean maternal age varied among study sites, while the mean gestational age did not.

Each site performed FISH analyses according to the instructions in the AneuVysion™ kit package insert. The percentage of aneuploid cells was determined by FISH after enumerating a minimum of 50 interphase nuclei per target; a minimum of 40 evaluable nuclei was deemed informative.

From this pivotal multi-center comparative study described above, the results of interphase FISH analysis were compared to standard cytogenetics.

True Aneuploid Cases

Among the 861 aneuploid cases, there were 75 +13; 192 +18; 322 +21; 107 45,X; 44 47,XXX; 57 47,XXY, 24 47,XYY; one -21; one XXY +18; one XXX +18; one 49,XXXXY; one 48,XXYY; one tetraploid 92,XXYY, and 33 triploids (19 69,XXX and 14 69,XXY) and one 46,XX,idic(18). Of which, 860 had % aneuploid cells by FISH greater than 60% and one 35%, which as mentioned earlier, was due to long storage. Thus, under the worst case scenario, for determination of true aneuploidy, FISH is able to detect 99.9% (860/861) of cases identified by standard cytogenetic analysis. Note also that one male and one female trisomy 21 fetal cases with mild maternal cell contamination were deemed informative.

Mosaic Cases
There were 62 true mosaic cases, as identified by standard cytogenetic analysis. There were 23 cases associated with X0, 10 with XXX, 8 with XXY, 7 with +21, 4 with +18, 2 with -18, 3 with +13, and 5 with X0 complexes. Even though aneuploid cell lines were detected in 60 cases, twelve of the 62 mosaic cases showed less than 10% % aneuploid cells by FISH, and 15, showed greater than 60%. The correlation of the % aneuploid cells is 0.76, between FISH assay and standard cytogenetic analysis.

Pseudomosaic Cases
There were 76 pseudomosaic cases, as identified by standard cytogenetic analysis. Among these 76 cases, 7 +13, 9 -13, one +18, 24 -18, 11 +21, 19 -21, 25 X0, one XXX, one XXY, one XYY, and 6 XXYY were observed. FISH assay results showed less than 10% aneuploid cells which is consistent with the euploid state.

Euploid Cases
Among 504 euploid cases identified by standard cytogenetic analysis, FISH also found each to have % aneuploid cells less than 10%. Thus, for determination of euploidy, FISH is able to detect 100% (504/504) of cases identified by standard cytogenetic analysis. Note also that all four male fetal cases with mild maternal cell contamination were deemed informative.

Pairwise Comparison between Cultured and Uncultured Specimens
There were 722 patients in the pivotal study with FISH assay performed on both uncultured and cultured samples of the same specimen.

Conclusions

Performance of AneuVysion is supported by the Vysis Quality Control Procedures and is demonstrated in the clinical and post-market studies. When the AneuVysion DNA Probe Kit is used as instructed in the package insert, the above statements describe its performance.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration
2098 Gaither Road
Rockville MD 20850

APR 1 3 2001

Russel K. Enns, Ph. D.
Vice President of Regulatory Affairs
Vysis, Inc
3100 Woodcreek Drive
Downers Grove, Illinois 60515

Re: K010288
Trade Name: AneuVysion® Multicolor DNA Probe Kit
Regulatory Class: Unclassified
Product Code: MAO
Dated: January 30, 2001
Received: January 31, 2001

Dear Dr. Enns:

We have reviewed your Section 510(k) notification of intent to market the device referenced above and we have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into either class II (Special Controls) or class III (Premarket Approval), it may be subject to such additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 895. A substantially equivalent determination assumes compliance with the Current Good Manufacturing Practice requirements, as set forth in the Quality System Regulation (QS) for Medical Devices: General regulation (21 CFR Part 820) and that, through periodic QS inspections, the Food and Drug Administration (FDA) will verify such assumptions. Failure to comply with the GMP regulation may result in regulatory action. In addition, FDA may publish further announcements concerning your device in the Federal Register. Please note: this response to your premarket notification submission does not affect any obligation you might have under sections 531 through 542 of the Act for devices under the Electronic Product Radiation Control provisions, or other Federal laws or regulations.

This letter will allow you to begin marketing your device as described in your 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801 and additionally 809.10 for in vitro diagnostic devices), please contact the Office of Compliance at (301) 594-4588. Additionally, for questions on the promotion and advertising of your device, please contact the Office of Compliance at (301) 594-4639. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR 807.97). Other general information on your responsibilities under the Act may be obtained from the Division of Small Manufacturers Assistance at its toll-free number (800) 638-2041 or (301) 443-6597 or at its internet address "http://www.fda.gov/cdrh/dsma/dsmamain.html".

Sincerely yours,

Steven Gutman

Steven I. Gutman, M.D., M.B.A.
Director
Division of Clinical Laboratory Devices
Office of Device Evaluation
Center for Devices and Radiological Health

Enclosure

510(k) Number (if known): K010288

Device Name: AneuVysion

Multi-Color DNA Probe Kit

Indications For Use:

The AneuVysion (CEP 18, X, Y-alpha satellite, LSI 13 and 21) Multicolor Probe Panel is intended to use CEP 18/X/Y probe to detect alpha satellite sequences in the centromere regions of chromosomes 18, X, and Y, and LSI 13/21 probe to detect the 13q14 region and the 21q22.13 to 21q22.2 region. The AneuVysion kit is indicated for identifying and enumerating chromosomes 13, 18, 21, X, and Y via fluorescence *in situ* hybridization (FISH) in metaphase cells and interphase nuclei obtained from amniotic fluid in subjects with presumed high risk pregnancies. It is not intended to be used as a stand-alone assay for making clinical decisions. FISH results are intended to be used as an aid in the diagnosis of numerical abnormalities of chromosomes 13, 18, 21 X and/or Y in conjunction with other information currently used in prenatal diagnosis, consistent with professional standards of practice [1]. This device is intended for use only with amniocyte cells; it is not intended for and has not been validated for use with other test matrices. This FISH assay will not detect the presence of structural chromosome abnormalities that can also result in birth defects. This FISH assay will be performed in cytogenetics laboratories.

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(Division Sign-Off)
Division of Clinical Laboratory Devices
510(k) Number

Concurrence of CDRH, Office of Device Evaluation (ODE)

Prescription Use ✓
(Per 21 CFR 801.109)

OR

Over-The-Counter Use ___

(Optional Format 1-2-96)